IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re the Application of Timothy P. Croughan — Examiner Kruse, D.

Serial No. 09/830,194

International Filing Date 05 November 1999 — Group 1638

35 U.S.C. § 371 Date 23 April 2001

For: Herbicide Resistant Rice — Atty. File 98A9-US Croughan

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

DECLARATION OF INVENTOR TIMOTHY P. CROUGHAN

STATE OF LOUISIANA

PARISH OF ACADIA

Timothy P. Croughan declares as follows

1.

I am the inventor of the above-identified patent application. I make this Declaration in support of that application.

CERTIFICATE

I hereby certify that this Declaration is being deposited with the United States Postal Service as first class mail in an envelope addressed to: Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450 on January 8, 2004.

John H. Runnels
Registration No. 33,451
January 8, 2004

2.

All experiments reported in the specification, and all experiments described in the present Declaration were conducted by me, or were conducted under my supervision.

3.

Field tests have been conducted to evaluate imidazolinone resistance of progeny from hybridizing PTA-904 with a diverse range of U.S. and foreign rice germplasm. The field tests, conducted at the Louisiana State University Agricultural Center's Rice Research Station in Crowley, evaluated herbicide resistance characteristics of hybrids between the herbicide resistant line PTA-904 (male parent) with each of ten different United States and sixteen different non-United States rice varieties (female parents). The diverse collection of foreign rice varieties originated from countries in Asia, Europe, Central America, and South America. Domesticated rice has two principal subspecies: *Oryza sativa indica* and *Oryza sativa japonica*. The varieties used in these field tests had approximately equal numbers of *indica* or *indica*-like varieties, on the one hand, and *japonica* or *japonica*-like varieties, on the other hand.

4.

The rice varieties used in these tests, together with their respective countries of origin, were as follows:

VARIETY	**COUNTRY OF ORIGIN**
Earl	**United States**
Bengal	
Cocodrie	
Cypress	
Drew	
Francis	
L202	

M202
9802051
Wells

Akitakomachi	**Japan**
Koshiakari	
CR1113	**Costa Rica**
CR5272	
Fedearroz 50	**Colombia**
Llanos 5	
CT408-6-F4-17-4	
Caribe 8	
Yacu 9	
IR64	**Philippines**
Guichow	**China**
Arborio	**Italy**
Carnaroli	
IRGA 369	**Brazil**
IRGA 416	
IRGA 417	

5.

The germplasm planted in these tests included F_2 lines, F_3 lines, F_5 lines, Backcross 1 F_2 (BC_1F_2) lines, BC_1F_3 lines, BC_1F_5 lines, BC_2F_2 lines, BC_2F_3 lines, BC_2F_5 lines, and BC_3F_2 lines.

6.

The tests included nearly 2800 rows, each one meter in length, as well as check rows of each of the 26 parental varieties and of PTA-904. One gram of seed (approximately 40 individual grains) was planted per row. Some of the check rows were sprayed with imidazolinone herbicide while others were left unsprayed as negative controls. The rice plants were sprayed with imidazolinone herbicide at the 4-5 leaf stage of development with 0.188 lb ai/A imazapyr (trade name Arsenal 2.0 AS) using a tractor mounted spray rig set to deliver 20 gallons per acre at 44 lbs/sq. inch pressure through VSV002 spray nozzles. One pint of crop oil surfactant was added per 20 gallons of spray mix. This herbicide application rate, 0.188 lb ai/A, was about three times higher than the more typical imidazolinone application rate of 0.063 lb ai/A.

7.

The effects of the imidazolinone application were determined 28 days after spraying. All check rows (i.e., each of the 26 non-resistant parents) sprayed with imidazolinone herbicide suffered 100% mortality, except that the PTA-904 rows showed no herbicide injury. The negative control check rows of wild type rice and PTA-904 rice that were left unsprayed also exhibited no injury symptoms. The PTA-904 rows that had been sprayed with imidazolinone herbicide were indistinguishable from those that had been left unsprayed, in striking contrast to 100% mortality in the imidazolinone-sprayed rows of non-resistant parental checks.

8.

Because the hybridized germplasm in the tests represented segregating populations at various degrees of homozygosity, individual rows typically contained small percentages of plants that were homozygous wild-type with respect to herbicide resistance. These plants exhibited the same susceptibility to the herbicide as their non-resistant parents, and were killed by the herbicide application. In striking contrast, the remaining resistant plants in each row exhibited no detectable

injury symptoms. Each of the hybrid rows derived from each of the twenty-six non-resistant parents contained numerous plants whose resistance to the imidazolinone herbicide (as applied in these tests) was indistinguishable from that of the resistant PTA-904 parent.

9.

A high degree of herbicide resistance was consistently observed in these tests across a diverse set of hybrids and backcrosses, despite the wide differences in genetic background found in the broad range of varietal parents used in these tests. At an elevated rate of application of a potent imidazolinone herbicide, all resistant hybrid lines in these tests performed equally well, exhibiting no injury symptoms from the herbicide application.

10.

All statements made in this Declaration of my own knowledge are true. All statements made in this Declaration on information and belief are believed to be true. These statements were made with the knowledge that willful false statements and the like are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the above-identified patent application or any patent issuing from that application.

Timothy P. Croughan

Timothy P. Croughan

January 8, 2004